# NSBRI Muscle Alterations and Atrophy Strategic Plan

## 7.0 MUSCLE ALTERATIONS & ATROPHY

**Team Leader:** Kenneth M. Baldwin, Ph.D.
Professor
Department of Physiology and Biophysics
University of California, Irvine
Medical Sciences I, Rm. D340
Irvine, CA 92697-4560
949-824-7192
949-824-8540 FAX
kmbaldwi@uci.edu

## 7.1 INTRODUCTION

Previous research involving both humans and animals clearly indicates that unloading the skeletal muscle system through prolonged space flight or bed rest causes a cascade of negative events within the body. Muscle mass is reduced and although the mechanism for this response is not known for certain, the atrophy is thought to be due to an imbalance between protein synthesis and protein degradation within the targeted fibers. Muscle strength is also reduced, leading to a decrease in physical performance and high power output capacity. However, the reduction in strength often exceeds the loss in muscle mass, suggesting that other, more complex mechanisms may be responsible for the reduced performance. A slow-to-fast shift in the contractile protein phenotype is observed, including shifts to faster myosin heavy chain (MHC) and calcium cycling proteins. These alterations induce the muscle fibers to become less economical in sustaining force output, resulting in an increased fatigability. This decreased resistance to fatigue is important because it could cause functional impairment that would affect the performance of extravehicular activity in space and emergency egress activity following landing. It is reasonable to suspect that the above changes will also make muscle more prone to injury than would otherwise be the case and that weaker muscles would result in an increased susceptibility to accidents that could cause damage to other systems, such as bone or connective tissue. Deleterious alterations in muscle properties may also be closely linked to changes in the ability of the nervous system to accurately control movements. All these effects could alter astronaut safety when performing any type of work in space.

## 7.2 RISKS

The following risks in the Muscle Alterations and Atrophy Discipline Area have been identified in the Critical Path Roadmap (risk number in parentheses):

- Loss of Skeletal Muscle Mass, Strength, and/or Endurance (28)
- Inability to Adequately Perform Tasks Due to Motor Performance, Muscle Endurance, and Disruption in Structural and Functional Properties of Soft and Hard Connective Tissues of the Axial Skeleton (29)
- Inability to Sustain Muscle Performance Levels to Meet Demands of Performing Activities of Varying Intensities (30)
- Propensity to Develop Muscle Injury, Connective Tissue Dysfunction, and Bone Fractures Due to Deficiencies in Motor Skill, Muscle Strength and Muscular Fatigue (31)
- Impact of Deficits in Skeletal Muscle Structure and Function on Other Systems (32)

Since several of these risks are operationally defined and interdependent, it is difficult to organize and develop a focused research program based on this set. Therefore, we have chosen to redefine the risks in this research area as follows:

- Loss of muscle mass, strength and endurance;
- Loss of motor control/movement performance due to changes in neural control;
- Proneness to muscle injury; and
- Impact of degeneration of muscle or increased injury of muscle on other systems such as bone and connective tissue.

This set of four risks underlies the risks listed in the Critical Path Roadmap and is less interdependent.

## 7.3 GOALS

The Muscle Alterations and Atrophy Team has the following goals for its program:

**Risk-Based Goals**

**Goal 1:** *Reduce risk of loss of muscle mass, strength and endurance*

**Goal 2:** *Reduce risk of loss of motor control/movement performance due to changes in neural control*

**Goal 3:** *Reduce risk of proneness to muscle injury*

**Non Risk-Based Goals**

**Goal 4:** *Develop monitoring methods using biochemical/molecular markers to predict potential anabolic and catabolic states in muscle*

**Goal 5:** *Develop rehabilitation methods (nutritional, pharmacological, and exercise-specific agents) that are effective in treating loss of muscle mass, strength and endurance*

**Goal 6:** *Develop Earth-based applications of exercise training paradigms to ameliorate problems of frailty, injury, morbidity, and mortality that are associated with the aging process, degenerative muscle disorders, and inactivity-related disorders*

**Goal 7:** *Integrate research and analysis*

## 7.4 DESCRIPTION AND EVALUATION OF CURRENT PROGRAM

The Muscle Adaptation and Alterations team (Muscle Team) will seek to reduce most but not all of the identified risks defined in section 7.2 above. In particular, we are not addressing the fourth of the risks listed in that section, dealing with the impact of muscle degeneration or injury on other systems. While it is apparent that there is important synergy between the properties of skeletal muscle and bone, the bone and connective tissue properties can be studied more effectively by the experts in the bone discipline field by exploiting models that affect both skeletal muscle and bone. The Muscle Team views Goal 1, Reduce risk of loss of muscle mass, strength and endurance, as its primary goal. This goal underlies the Team's research program to prevent or minimize the deleterious adaptations of the structure and function of skeletal muscle in response to the prolonged states of unloading occurring in space flight. Importantly, the amelioration of these deficits will have an inherent beneficial effect in reducing the other, secondary, risk-based goals (Goals 2 and 3) that concern the motor control of muscle function and/or movement performance and the reduction of the proneness of weakened skeletal muscle to injury. Due to budgetary constraints that limit the current size of the investigative team, these two goals, though important, will not be a major focus at the present time. Future research efforts in addressing these secondary goals will evolve as the program is expanded. Additional non risk-based goals include those associated with the assessment of health and application of medical care, transfer of information to Earth-based applications, and facilitation of integration of research and analysis.

In 2001, the Muscle Team's research program was totally restructured. The current team is comprised of ten principal investigators, seven of whom are new NSBRI investigators. The restructuring involved a significant refocus and a shift in the team's research objectives. Table 7.1, entitled "Current Project Research Activities," summarizes for each current Muscle Alterations and Atrophy Team project those risks that are currently being addressed, the experimental system, the countermeasure target and whether a project is part of the strategic steps of Phase 1, 2 or 3 Activities.

All projects directly or indirectly address the critical problem of muscle atrophy and the corresponding loss in muscle strength and human performance. The Baldwin, Goldberg, and Antin projects seek a better understanding of the mechanisms associated with the imbalance in protein synthesis and protein degradation. The Goldberg and Antin projects, while addressing mechanisms of degradation, focus on different, but complementary processes that impact protein loss. The Antin project examines calpain and its role in the regulation of the rate of muscle protein accumulation and the potential use of inhibitors of calpain mechanisms as countermeasures in animal and cellular models. The Goldberg project, alternatively, uses animal and human models to seek to clarify the basis of activation of the ubiquination pathway in unloaded muscles and whether inhibitors of this pathway may be a countermeasure to muscle atrophy. The Baldwin project uses an animal model to directly examine the switch in muscle protein phenotypes that is associated with muscle unloading and to develop a resistance exercise program that ameliorates muscle atrophy and prevents phenotype switch in response to space flight. In addition, Baldwin's group is interacting with scientists outside the NSBRI, such as Dr. Suzanne Schneider at the University of New Mexico and Dr. Per Tesch at the Karolinska Institute, who are directing research projects that seek resistance training countermeasures to prevent muscle atrophy in human subjects in response to ground based models of unloading.

The Kandarian (rodent) and Hamilton (human) projects are determining which key genes are involved in both atrophy and hypertrophy processes using functional genomics and global transcriptional profiling. By examining the expression of ~8,000 genes simultaneously over a time course of unloading, the Kandarian project will uncover patterns of gene expression underlying muscle atrophy. By using a variety of clustering approaches and by comparing the gene expression data set to that in the literature from hypertrophied, aging, and cachextic muscle (Goldberg lab), clusters of gene expression unique to muscle unloading will be revealed. These data will also be compared to the human dataset produced by the Hamilton project. The genes and gene clusters that are uniquely altered by disuse will be studied in further detail (by various investigators) to determine their role during disuse/unloading atrophy. Work recently published from this lab showed that unloading activated an NF-kB pathway, so the effect of NF-kB inhibitors (e.g., asprin, curcumin) will be tested for their ability to ameliorate atrophy and reverse the affected gene clusters.

Focusing mainly on $Ca^{2+}$-sensitive processes, the Wiseman project will provide insight into cell signaling and regulatory factors that control the protein phenotype and the metabolic capacity of isolated muscle cells. Direct manipulation of cytosolic $Ca^{2+}$ levels may stave off changes in muscle upon unloading. The Reid project will dissect the role of both ionizing radiation and reactive oxygen species on mechanisms of muscle fatigue, as well as muscle atrophy processes in animals and cultured cells. Antioxidants will be tested as potential countermeasures to these effects in human subjects. The Sinha/Edgerton project will use humans to dissect the effects of unloading on stress/strain function in skeletal muscle and determine how muscles may become prone to injury in the face of atrophy and loss of strength. It will also examine its MRI technology for potential use for evaluation of exercise countermeasures using the human lower limb suspension model. Additional interactions are evolving among the Goldberg, Sinha/Edgerton and Baldwin projects, as well as between the Hamilton and Sinha projects. These groups aim to establish "ground zero baselines" that define the mechanisms of muscle atrophy by using the spinal isolation model where animals have complete inhibition of neuromuscular activity.

Complementing the above projects are two additional projects that were originally part of the Integrated Human Function Team. However, due to restructuring within NSBRI program objectives, two projects were reallocated to the Muscle Team, since they were clearly congruent to the goals of the Muscle Team as outlined above.

The Kushmerick project uses a combination of non-invasive $^{31}P$ and $^{1}H$ NMR spectroscopies, MR and ultrasound functional images, biomechanical analyses and multi-level modeling in order to conduct analyses leading to an integration of the metabolic and mechanical mechanisms of human muscle. Analysis of limb function is crucial to plan training and to select personnel for optimal efficiency and economy with minimal risk and fatigue. The basic science of this proposal evaluates the mechanisms responsible for transient and steady state performance of limb muscle, which is critical for astronaut performance. This analysis requires the specification of: 1.) the mechanical power output by specific muscles during limb functions; 2.) the analysis of the properties of different muscles in the same individual and of the same muscles in different individuals; 3.) the partition of energy demand into mechanical output and ion transport costs; 4.) the division of metabolism quantitatively between glycolytic and oxidative processes and analysis of their inter-related controls; and 5.) the relationship between these intracellular and mechanical properties and muscle blood flow and perfusion. These experimental approaches and information are crucial to develop a model-based approach to the study of *in vivo* muscle energy balance in humans because the relevant data is not available and more importantly, and because the conceptual basis for integrating the component cellular mechanisms can only be evolved from these new observations.

The Chase project, while originally targeted to the Integrated Human Function Team also provides an excellent fit with the existing Muscle Team. The overall goal of this project is to produce a muscle cell model (digital cell) that will: explain biomechanical adaptations that occur with alterations in muscle protein isoforms due to changes in activity level; predict bioenergetic changes associated with changes in activity level; and be integrated into computational models of human limb and heart. The essential molecular and subcellular components of the model will be identified and algorithms constructed based on experimental data obtained in a controlled environment. The cell model will be tested against published biomechanical and bioenergetic data obtained under a broad spectrum of environmental conditions. The muscle cell model will be one of the main building blocks for constructing a model of integrated human function because the cell is the basic unit of physiological organization and because the musculoskeletal system is ~80% of body mass, thus a major determinant of energy consumption, and is responsible for movement and cardiovascular function. This particular project will closely interface with both the Kushmeric projects and the Wiseman projects as described above.

We anticipate that as the ground-based research matures, over time, three fundamental counter-measure strategies will be applied to ameliorate skeletal muscle dysfunctions. These include:

1) Different forms of preflight and inflight physical exercise with activity-unique prescriptions:
    - *A resistance training prescription to maintain muscle mass and strength.*
      Studies conducted to date on both animals and humans clearly suggest that exercise paradigms of the high resistance type are only partially effective in reducing muscle atrophy. Future research needs to: a) Utilize human experiments to define the success of resistance paradigms using ground-based models that induce atrophy (bed rest, limb suspension). This effort should be carried out by interactions both within and outside the muscle team. b) Understand the mechanism(s) behind the partial effectiveness of resistance training. c) Define a better exercise prescription that is more effective in reducing muscle atrophy, as well as, more economical in terms of the time devoted to performing such a countermeasure on a daily basis.
    - *An aerobic exercise paradigm that would improve both cardiovascular fitness and skeletal muscle endurance.*
      Aerobic exercise is an important countermeasure for both skeletal muscle and cardiovascular endurance. While it is recognized that activities such as running and cycling are effective in enhancing endurance in normal weight bearing modes, it remains to be determined whether these paradigms are effective when used with individuals experiencing chronic states of unloading even when performed in conjunction with resistance training paradigms. Activities carried out with both human (Hamilton) and animal models (Baldwin) will be developed in the next few years to provide insight into this issue.
    - *An activity paradigm that would specifically target the sensory-motor pathways to maintain posture, balance and locomotor skills.*
      This type of activity is an important part of a total exercise prescription, and future research projects need to be sought to address this issue using new funding initiatives.
    - *An impact-loading paradigm that could conceivably affect both the skeletal muscle system and the skeletal systems to stimulate/maintain bone homeostasis.*
      While it has been recognized that stress/strain reaction forces impact both muscle and bone, research addressing whether there are synergistic/interactive effects of bone stress on muscle and vice versa is still in the infant stages. A visionary goal of the muscle team is to address this problem in future research projects. This research could be facilitated by interactions with both the Bone and Nutritional Teams.

2) Human-powered artificial gravity (gravity-equivalent exercise).

Funding augmentation should be sought to begin studies using artificial gravity (human-powered centrifuge) as an alternative countermeasure strategy. These activities need to be a high priority for flight testing, because this activity paradigm has the potential to encapsulate all four of the above mentioned exercise-type prescriptions and positively add to the bone, cardiovascular, vestibular, and nutritional/fitness countermeasure strategies. It is envisioned that the Muscle Team will take a lead role and undertake a multifaceted, integrative research project involving the Neurovestibular, Cardiovascular, Bone, and Nutrition/Physical Fitness research Teams to address these fundamental overarching physiological problems using artificial human-generated gravity equivalent exercise as the centerpiece in the countermeasure program.

3) Novel nutritional, pharmacological and hormonal/growth factor approaches

These activities are being explored in the current funding period. In particular, the gene chip analyses currently underway will contribute significantly to the collective research effort.

In the future, as more resources become available, we would like to seek funding for projects which address the relationship of muscle tissue changes with actual movement performance and that strive to differentiate between problems associated with neural control versus muscle tissue effects (Goal 2). Additional projects examining the proneness of weakened muscle to injury should also be sought (Goal 3). These projects will build off of the fundamental knowledge generated by the combined Sinha, Kushmeric and Chase projects.

Some progress has already been made in achieving the first of the non risk-based goals, e.g., Goal 4, "Develop monitoring methods using biochemical/molecular markers to predict potential anabolic and catabolic states in muscle"**.** In the current funding period, several genes and their encoded proteins have been identified that could play key roles in muscle homeostasis and that could be used as markers. For example, Goldberg's team has identified a novel gene, termed atrogin 1, that may play a pivotal role in regulating muscle-wasting disorders. The Kandarian team has revealed 16 different genes encoding proteasome subunits and 5 ubiquitination genes (including atrogin-1) that are upregulated with unloading, but the ubiquitination genes are upregulated earlier and to a greater magnitude than the proteasome genes. This effect is a distinctly different catabolic response to that seen with aging, for instance. This example is one of many examples of differentially expressed gene clusters revealed by the Kandarian project. Also, the Baldwin group has shown that atrophying skeletal muscles of rodents and humans initially undergo marked losses in ribosomal RNA that serve as the machinery for translating the protein necessary for maintaining muscle mass. Additionally, growth factors (IGF-1 and mechano growth factor) have been identified that appear to be essential in turning on anabolic processes and inhibiting catabolic states in muscle undergoing an altered loading state. Thus, it is anticipated that a cadre of molecular markers will be identified that can be used to assess the state of the muscle in astronauts. By controlling the expression of the genes for these important proteins, we may also be able to maintain the muscle from one functional state to another. In the future, the monitoring methods that we develop for use with the astronauts will also have direct applicability for addressing a variety of Earth-based problems associated with muscle disorders due to inactivity, disease, and sports injury.

Two other non risk-based goals (Goals 5 and 6) are important aims of the Muscle Team's research mission. The future vision of the program will involve expanding the knowledge of muscle cell structure and function and the operational activity regimens so that we can best design rehabilitation methods that overcome the muscle deficits that occur in astronauts in response to prolonged space flight (Goal 5) and the well-known effects of aging on skeletal muscle that closely mimic these effects (Goal 6). Regimens similar to the successful preflight training paradigms may likewise be used as preventative measures for the effects of aging on muscle. Similar preventative and rehabilitative methods may also aid a variety of muscle degenerative disorders and inactivity-related diseases (e.g. type II diabetes) that severely impact large populations of individuals. Thus, Earth-based applications addressing problems of frailty, injury, morbidity, and mortality that are associated with Earth-based disorders represent an important outcome of the Muscle Program. To these efforts, the expertise currently on board the muscle Team (Sinha, Kushmerick, Hamilton, and Chase Projects), should play a significant role in advancing Goals 5 and 6.

Another important non risk-based goal of the Muscle Team (Goal 7) will be to enhance the interaction of individual Muscle Team investigators a) among the current team's research infrastructure, b) among investigators within other teams (Bone, Nutrition and Fitness, Neurovestibular, Cardiovascular), and c) with investigators not formally associated with the NSBRI. This strategy should provide an effective means to leverage a greater scientific return relative to the resources invested by the NSBRI. Table 7.2 summarizes our current efforts at integration. A few examples of integration of the Muscle Team with other teams or researchers outside of the NSBRI are as follows: Dr. Baldwin currently serves as a co-investigator on Dr. Shapiro's Bone Team research project that investigates the effects of bisphophonates on the integrity of bone and skeletal muscle. He is currently interacting with Dr. Ann Kennedy of the radiation team in testing a novel protease inhibitor for its properties in preventing muscle wasting in conjunction with resistance exercise using rodents as the model system. Also, Dr. Baldwin is collaborating extensively with investigators external to the NSBRI program in seeking countermeasures to muscle atrophy in human models of atrophy (both bed rest and limb suspension models). These interactions are with Dr. Suzanne Schneider at the University of New Mexico and with Dr. Per Tesch at the Karolinska Institute in Stockholm, and these projects are using novel resistance training devices and innovative training programs that are aimed at preventing muscle atrophy. As another example of integration from the Muscle Team, the modeling work of Dr. Kushmerick and Dr. Chase will serve to integrate the team with the other NSBRI teams as part of the Core Integrated Human Function effort.

## 7.5 OBJECTIVES AND STRATEGIC ACTIVITIES

Presented here are the objectives underlying each goal and the strategic activities that we plan to use to achieve the goals and objectives of our program. Table 7.3 summarizes the timeline of the Muscle Team's strategic activities for Goal 1. The other goals are still being developed and will not be summarized in a similar table.

**Goal 1:** *Reduce risk of loss of muscle mass, strength and endurance*
<u>Objective 1A.</u> Assess risk and target level of acceptable risk

- Complete activities to achieve this objective which are currently a part of the projects in which Dr. Baldwin's group is interacting with outside investigators using resistance training with bed rest studies (in collaboration with Dr. Schneider) as well as using human models of limb suspension in combination with concentric/eccentric resistance exercise (in collaboration with Dr. Tesch).

Objective 1B. Determine mechanisms

- Complete projects defining the underlying processes that cause the catabolic muscle state and develop a better understanding of the processes regulating muscle protein degradation (Antin, Goldberg, Kandarian, Hamilton, and Baldwin projects).
- Complete projects defining the mechanism of the slow-to-fast shift in contractile protein phenotype (e.g., shifts to faster myosin heavy chain and calcium cycling proteins) upon muscle unloading (Wiseman and Chase projects ).
- Complete project that seeks insight into the role that reactive oxygen species play in causing muscle atrophy processes (Reid project).
- Complete projects defining muscle loading-sensitive genes using both animal and human subjects with the intent to ultimately determine new approaches to regulating protein balance in skeletal muscle (Hamilton and Kandarian projects).
- Complete projects that address the stress- strain relationship in skeletal muscle of subjects with tendon injuries and muscle weakness due to atrophy processes; and assess the metabolic states of muscle in performing different types of activity (Sinha/Edgerton and Kushmerick projects).
- Initiate a project that determines effects of artificial gravity (such as human-powered centrifuge) on muscle. (This possibility depends on resources coming available by NASA, NSBRI, and NIH.)
- Initiate more projects in humans exploring the reduction of muscle atrophy by resistive exercise.

Objective 1C. Develop countermeasures

- Complete current animal and human projects with collaborators outside of NSBRI (Baldwin Project*)* and initiate new projects in humans defining and testing potential preflight and inflight activity paradigms (such as different types of resistive exercise training) that create an anabolic state in muscle, ameliorating atrophy processes. Expand to involve the bed rest model and include nutritional interventions (antioxidants and amino acid supplements, etc.) in collaboration with the Nutrition and Fitness Team (see below under Integration Goal).
- Complete current projects that seek pharmacological interventions that can blunt the processes of muscle protein degradation or other effects of muscle unloading (Antin, Kandarian, and Goldberg projects.) Expand to involve co-examination of exercise and pharmacological agents as joint countermeasures.
- Complete current study on the role of antioxidants as a nutritional countermeasure strategy to muscle atrophy (Reid project). This project could evolve to include projects in which amino acid supplements and antioxidants are used in combination with resistance exercise studies involving the animal model of Baldwin's group.
- Initiate studies to determine whether artificial gravity can be a good alternative countermeasure strategy for muscle degeneration.

**Goal 2:** *Reduce risk of loss of motor control/movement performance due to changes in neural control*

Objective 2A. Assess risk and target level of acceptable risk

Objective 2B. Determine mechanisms

- Seek new funding initiatives and initiate studies that determine the relationship of muscle tissue changes with actual movement performance and differentiate mechanisms associated with neural control from muscle tissue effects that could change the ability of the nervous system to accurately control movements and regulate the properties of muscle strength.

Objective 2C. Develop countermeasures

**Goal 3:** *Reduce risk of proneness to muscle injury*

Objective 3A. Assess risk and target level of acceptable risk

Objective 3B. Determine mechanisms

- Complete study to determine unloading sensitive genes in animals and humans that target inflammatory processes and signaling pathway molecules *(*Kandarian and Hamilton projects).
- Complete study examining stress-strain in muscle during atrophy and recovery (Sinha/Edgerton and Kushmerick project*)* and initiate new studies to amplify how resistance loading and unloading affects stress-strain reactions in human subjects.
- Seek new funding opportunities and initiate studies addressing the mechanism underlying muscle weakness, fatigue, and the proneness of weakened muscle to injury (Kushmerick, Chase, Wiseman, Reid, and Sinha/Edgerton /projects).

Objective 3C. Develop countermeasures

*Note: As discussed elsewhere in this document, while Goals 2 and 3 are important objectives of the Muscle Team, they are currently not a high priority due to insufficient resources available to cover these topics. As resources evolve, these goals will receive a greater priority.*

**Goal 4:** *Develop monitoring methods using biochemical/molecular markers to predict potential anabolic and catabolic states in muscle*

Objective 4A. Identify key proteins

- Complete experiments to identify key molecular markers and cellular processes that regulate protein balance in skeletal muscle via protein transcriptional/translational and degradative pathways *(*Baldwin, Goldberg, Kandarian, Antin projects).

Objective 4B. Develop methods to observe key proteins

**Goal 5:** *Develop rehabilitation methods (nutritional, pharmacological, and exercise- specific) that are effective in treating loss of muscle mass, strength and endurance*

Objective 5A. Identify effective rehabilitation methods

- Complete research on both animal models and humans using novel resistance loading paradigms and prescriptions that utilize different contraction modes (isometric, concentric, eccentric).
- Seek funding to initiate acute studies that characterize ground based adaptive responses to intermittent bouts of hypergravity-induced stimuli via use of a human-powered centrifuge facility at University of California Irvine.

Objective 5B. Develop and test specific rehabilitation prescriptions

**Goal 6:** *Develop Earth-based applications of exercise training paradigms to ameliorate problems of frailty, injury, morbidity, and mortality that are associated with the aging process, degenerative muscle disorders and inactivity-related disorders.*

Objective 6A. Identify partners for Earth-based applications

- Seek funding with other agencies that conduct research into medical concerns that parallel the muscle deficits that occur in astronauts in response to prolonged exposure to microgravity.

Objective 6B. Work with partners to make exercise paradigms available to needy Earth-based populations

**Goal 7:** *Integrate research and analysis*
Objective 7A. Integrate research within the Muscle Alterations and Atrophy Team.

- Continue current integration efforts among the Muscle Team investigators as summarized in Table 7.2.

Objective 7B. Integrate research with other teams, using modeling as well as other approaches.

- Initiate in-depth studies, in conjunction with other NSBRI teams, of the effects of artificial gravity on skeletal muscle structure and function and other affected systems in microgravity. These studies will also make use of the unilateral limb suspension model.
- Explore interactions with the Nutrition and Fitness Team concerning how the separate and combined effects of nutritional modification and physical exercise impact muscle homeostasis and protein balance in bed rest subjects.
- Work with NSBRI Bone Team to begin co-examination of effect of muscle degeneration on other systems. Continue interactions with the Bone Team on countermeasures to both bone and muscle wasting using bisphosphonates, nutrition, and loading paradigms on spinally injured patients. Interact with the Bone Team on using spinally injured subjects as models to study the restoration of wasted muscle through electrical stimulation of muscles and the possible co-maintenance of bone mass. These studies could also explore the combined interventions of bisphosponates and mechanical stress on muscle and bone homeostasis.
- Continue modeling work towards the Core Integrated Human Function effort (Chase, Kushmerick)

Objective 7C. Integrate research with investigators not formally associated with the NSBRI

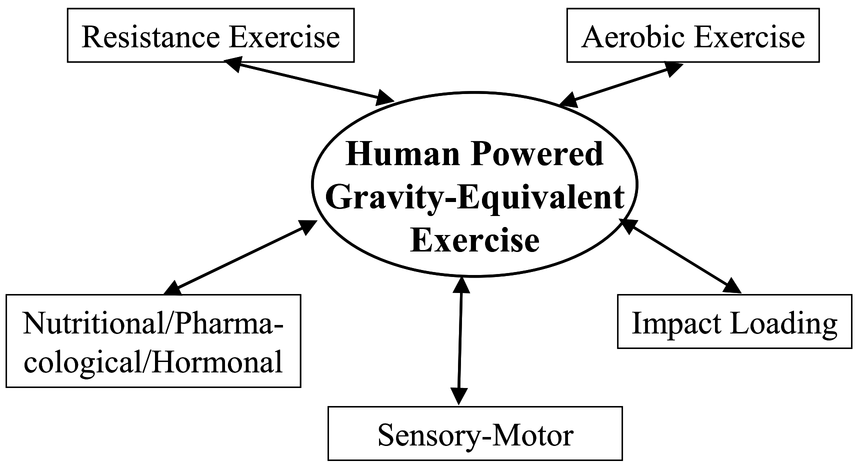
- Continue interaction of Baldwin's group with scientists outside the NSBRI, such as Dr. Suzanne Schneider at the University of New Mexico and Dr. Per Tesch at the Karolinska Institute, who are directing research projects that seek resistance training countermeasures to prevent muscle atrophy in human subjects in response to ground based models of unloading

## 7.6 SUMMARY

In the next 3-5 year time span, the Muscle Team should be able to successfully implement both its fundamental (mechanistic) and applied research programs that address the identified Risks outlined in this Research Plan. As discussed in the plan, the primary goal initially will be to focus on "reducing the risk of loss of muscle mass, strength and endurance". New insights will be derived concerning the cellular and molecular mechanisms of the muscle atrophy process, as well as, proposals of exercise paradigms that are mechanistic in terms of maintaining positive protein balance in skeletal muscle. In addition, the team will evolve a strategy of identifying and validating the ability of different exercise paradigms used in conjunction with nutritional and pharmacological interventions to ameliorate the loss of muscle function (mass, strength and endurance) that occurs in response to chronic states of unloading. Furthermore, Earth-based benefits will be generated both in the prevention of and treatment of a variety of inactivity- and aging-related disorders that are associated with muscle dysfunction, as well as, other disorders (e.g., type II diabetes) that are related to disuse and loss of skeletal muscle integrity.

We also envision, through the development and testing of a human-powered gravity equivalent countermeasure device, an overarching countermeasure paradigm that has the potential, when used in combination with other countermeasures strategies, such as nutritional and pharmacological therapies, to not only ameliorate muscle dysfunction, but to significantly maintain the homeostasis of the skeletal (bone), vestibular, and cardiovascular systems. The integrity of each of these other systems is also compromised by prolonged exposure to unloading states. These strategic cross cutting interactions both within the Muscle Team and with other investigative teams are illustrated in Figure 7.1 below and in the tables presented at the end of the Muscle Team Plan.

**Fig. 7.1:** The Figure below illustrates the vision of the Muscle Team as to how the human-powered centrifuge could serve an overarching training device. When used in combination with other countermeasure strategies, this device could, in addition to ameliorating the identified risks of the skeletal muscle system, reduce deleterious alterations that impact the functional integrity of vestibular, cardiovascular, and skeletal systems.



**National Space Biomedical Research Institute**
**MUSCLE ALTERATIONS AND ATROPHY PROGRAM**

## Table 7.1. Project Research Activities

| PI/Project | Risk(s) Addressed | Countermeasure Target | Experimental System | Phase 1 Activities: Focused Mechanistic Research | Phase 2 Activities: Preliminary Countermeasure Development Research | Phase 3 Activities: Mature Countermeasure Development Research |
|---|---|---|---|---|---|---|
| **ANTIN**/Calpains in Simulated Microgravity-Induced Atrophy | Loss of mass, strength and endurance | Not Applicable | • Hindlimb-unweighted transgenic mice<br>• Cultured L8 muscle cells | Understand muscle protein degeneration | Test protective effects of calpastatin in transgenic mice. | |
| **BALDWIN**/Role Muscle Loading on Mechanisms of Protein Translation and Impact on Unloading-Induced | • Loss of mass, strength and endurance<br>• Muscle injury | Resistance exercise training | Hindlimb-unweighted rat | • Understand muscle protein degeneration<br>• Determine slow-to-fast phenotype shift | Test activity paradigms that create anabolic state and reduce atrophy | Interact with external researchers on countermeasures with human bed rest studies |
| **Chase/C**ell and Molecular Biomechanics: Cardiac and Skeletal Muscle | Loss of mass, strength and endurance | Integrative Modeling (Integrated Human Function Core, "Digital Human") | • High vs. low-activity rats; computational models;<br>• indlimb suspension | • Focused mechanistic research: phenotype biomechanics of cells; model bio-mechanics of cells | Adapt phase I model of animal cells to human muscle cells | Integrate cell biomechanics model into "digital human"; incorporate muscle adaptation mechanisms |
| **GOLDBERG**/Activation of Protein Breakdown in Muscle Upon Unloading and Possible Countermeasures | Loss of mass, strength and endurance | Pharmacological and activity factors in altering protein degradative processes | Different models of muscle wasting | Understand the role of atrogin-1 in muscle wasting | Pharmacologic interventions in muscle wasting | Determine role of inhibitors of muscle wasting on atrophy processes in response to unloading states |
| **HAMILTON**/Genomics of Human Skeletal Muscle During Bed Rest and Exercise | • Loss of mass, strength and endurance<br>• Muscle injury | Resistance and endurance exercise training | Bedrest and limb unloading | Identify the loading responsive genes; validate results using bioinformatics and comparative models | • Test activity paradigms to prevent metabolic and atrophic changes.<br>• Use genomics to select "nonresponders" to unloading | • Screen subjects for genetic selection of resistance to unloading.<br>• Integrate exercise, nutritional and pharmacological measures. |

## Table 7.1. Project Research Activities (continued)

| PI/Project | Risk(s) Addressed | Countermeasure Target | Experimental System | Phase 1 Activities: Focused Mechanistic Research | Phase 2 Activities: Preliminary Countermeasure Development Research | Phase 3 Activities: Mature Countermeasure Development Research |
|---|---|---|---|---|---|---|
| **KANDARIAN**/Gene Expression Profiling of Unloaded Skeletal Muscle | Loss of mass, strength, and endurance | Pharmacological NF-kB pathway e.g. aspirin, curcumin | Hindlimb unloaded rats and mice | Transcriptional markers of catabolic state –(i.e., unloading atrophy) | Test if aspirin alleviates atrophy (by inhibition of NF-kB pathway) | Examine gene expression profiling on tissue (other investiga-tors) on which counter-measure was done |
| **KUSHMERICK**/ Integrating human muscle energetics and mechanics | • Loss of muscle mass, strength and endurance<br>• Muscle injury | • Exercise<br>• Monitoring and diagnostic procedure<br>• Integrative Modeling (Core) | Human leg and hand muscle | • Define normal limits of energy balance<br>• Determine acceptable level of metabolic and energetic risk | • Individualize physiologic and energetic parameters<br>• Test efficacy of exercise protocols | |
| **REID**/Redox Modulation of Muscle Function in Microgravity | Loss of mass, strength and endurance | Antioxidants, nutritional supplements | • Cultured myocytes,<br>• Excised muscle,<br>• Hindlimb-unloaded mice<br>• Humans | Evaluate signaling pathways that regulate catabolism | Test countermeasures for muscle wasting, weakness in unloaded mice | Test countermeasure for handgrip fatigue in humans |
| **SINHA**/In-Vivo Stress-Strain Dynamics in Human Muscle | • Loss of mass, strength and endurance<br>• Muscle injury and atrophy | Exercise (rehabilitative) | MRI and muscle function | Understanding stress/strain properties of muscle so can reduce injury and improve muscle strength | Examine atrophy processes on stress/strain properties | Better techniques to evaluate muscle proneness to injury and rehabilitative processes |
| **WISEMAN**/Ca++ Homeostasis and Muscle Phenotype: Role of Cellular Energetics | Loss of mass, strength and endurance | • Pharmacological<br>• Nutrition/Diet | • Isolated muscles<br>• Intact hindlimb | • Understand role of energetics and altered activity on calcium handling in mitochondria and sarcoplasmic reticulum<br>• Understand fast-slow transition phenotype switching | • Test therapeutics on calcium handling<br>• Use model to test phenotype switch | |

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## Table 7.2. Integration Activities

| | ANTIN | BALDWIN | CHASE | GOLDBERG | HAMILTON | KANDARIAN | KUSHMERICK | REID | SINHA | WISEMAN |
|---|---|---|---|---|---|---|---|---|---|---|
| **Internal Communication** | • Reid<br>• Wiseman<br>• Hamilton<br>• Kandarian | • Goldberg and Sinha<br>• Shapiro (Bone)<br>• Lupton (Nutrition &Fitness) | • Kandarian,<br>• Wiseman,<br>• Kushmerick,<br>• Bers, Coolihan, McCulloch (Cardio)<br>• Cabrera (Nutrition) | • Baldwin,<br>• Sinha/ Edgerton<br>• Kandarian | • Antin<br>• Sinha<br>• Baldwin<br>• Kandarian | • Hamilton<br>• Goldberg<br>• Antin<br>• Reid | • Sinha<br>• Chase<br>• Cabrera in Nutrition<br>• Bers, Coolahan McCulloch (Cardio) | • Antin<br>• Wiseman<br>• Goldberg<br>• Jones (JSC)<br>• Butel, Conner (Immune) | • Baldwin,<br>• Hamilton,<br>• Goldberg | • Antin<br>• Reid<br>• Chase<br>• Kushmerick |
| **Integrated Experiment Development** | • Transgenic animals/ Reid | • Spinal injury (Bone)<br>• Resistance exercise protocols (Nutrition) | Cellular energetics (Wiseman) | • Baldwin/ Sinha/ Edgerton;<br>• Hamilton/ Kandarian | | | Exercise protocols | • Hindlimb unloading<br>• Transgenic mice<br>• Handgrip fatigue | TBD | • Chronically stimulated hindlimb<br>• Hindlimb suspended animals |
| **Sample Sharing** | • Transgenic animals for physiological studies/ Wiseman, Reid | • Muscle biopsies/ Shapiro (Bone)<br>• Muscle samples / Goldberg, Kandarian, Hamilton | • Model Component (Kushmerick)<br>• Hindlimb suspension projects (Baldwin) | • Baldwin;<br>• Sinha/ Edgerton | • Muscle biopsies/ Sinha<br>• Muscle samples/ Baldwin<br>• Other ongoing human studies | Samples from Baldwin and other PIs performing atrophy counter-measures | none | • Probes for transgene develop./ Antin<br>• Muscles from irradiated mice/ Butel, Conner, Gridley | • Hamilton<br>• Baldwin | • Reid<br>• Antin (Potentially Kandarian) |

## Table 7.2. Integration Activities (continued)

| | ANTIN | BALDWIN | CHASE | GOLDBERG | HAMILTON | KANDARIAN | KUSHMERICK | REID | SINHA | WISEMAN |
|---|---|---|---|---|---|---|---|---|---|---|
| **Synergistic Studies of Opportunity** | | • Planning of human-powered centrifuge (Bone, Fitness, Cardio, Neurovestib)<br>• Resistance exercise training with humans /Schneider and Tesh (external)<br>• Bisphosp ho-nates/ Shapiro (Bone)<br>• Pprotease inhibitors &muscle atrophy responses (Radiation)<br>• Lupton (Nutrition) | N/A | Combined studies on muscle wasting via hindlimb suspension and pharmacologic interventions | • Model of the entire human genome during unloading<br>• Genomic screens as a test to identify novel gene targets for CMs<br>• Compare CMs on muscle metabolism and gene expression<br>• CM model: Compare rat and human responses to similar CMs & unloading | | • Compare generality of human and animal training results<br>• Test "energy phenotype" with adapative responses to exercise<br>• Integrate blood flow, mechanics and energetics with training | • Muscle response to gamma irradiation/ Butel, Conner, Gridley<br>• Muscle-specific, inducible transgenic mice/ Antin, Wiseman<br>• Unloading effects on calcium regulation and metabolism / Wiseman<br>• Beta testing of NASA device for handgrip evaluation on ISS / Jones (JSC) | • Baldwin, Project<br>• Hamilton project | • NASA funded bone group at MSU developing model of mechani-cally altered limbs (unloaded and loaded)<br>• Cell signaling group at MSU is collabora-tion on calcium measure-ments in muscle |
| **Development of Computer Model of Integrated Human Function** | | TBD | Muscle Cell and Molecular Biomechanics for Integrated Human Function Core | | Input data from the entire human genome for muscle and other tissues | | Integration of muscle modeling effort with cardiovascular and nutrition | | TBD | |

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## Table 7.3. Achieving Goal 1: Reduce Risk of Loss of Muscle Mass, Strength and Endurance

| Countermeasure Development Phases | Pre 2001 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Phase 0: Observational & Phenomenological Research** | | | | | | | | | | | | | |
| **Phase 1: Focused Mechanistic Research** | | | | | | | | | | | | | |
| • Understand muscle protein degradation<br>• Determine slow-to-fast phenotype shift<br>• Discern role of reactive oxygen species<br>• Define loading-sensitive muscle genes | | | | | | | | | | | | | |
| • Determine how resistive exercise reduces atrophy of human muscle<br>• Determine effects of artificial gravity on muscle<br>• Identify acceptable target levels of risks in humans | | | | | | | | | | | | | |
| **Phase 2: Preliminary Countermeasure Development Research** | | | | | | | | | | | | | |
| • Test activity paradigms that create anabolic state and reduce atrophy in animals and humans<br>• Test pharmacological interventions for muscle degradation and other muscle unloading effects<br>• Study role of antioxidants as nutritional countermeasure strategy | | | | | | | | | | | | | |
| • Determine whether artificial gravity is a feasible countermeasure to muscle atrophy in humans | | | | | | | | | | | | | |
| **Phase 3: Mature Countermeasure Development Research** | | | | | | | | | | | | | |
| • Develop integrated exercise, nutritional, and pharmacological countermeasure and test in humans | | | | | | | | | | | | | |
| • Determine whether artificial gravity, in conjunction with the exercise, nutritional, and pharmacological countermeasure above, further reduces muscle atrophy in humans | | | | | | | | | | | | | |
| **Phase 4: Countermeasure Evaluation & Validation** | | | | | | | | | | | | | |
| • Testing of integrated exercise, nutritional, pharmacological countermeasure with artificial gravity | | | | | | | | | | | | | |
| **Phase 5: Operational Implementation of Countermeasure Strategy** | | | | | | | | | | | | | |